Updated toxicity test protocol for larval Pacific oyster ATT 1.docx

Main document changes and comments

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NOTE TO READERS: This version of the tox test protocol builds on what we learned from the successful first test with oyster larvae. The two major changes are to the exposure chambers, and the samples for routine water chemistry (pH, D.O., salinity, temperature). Larvae were much more visible in the frosted white plastic cups than they were in either glass beakers or plastic petri dishes, which will make them easier and quicker to count during the test. We need to place more than 50 mL water in beakers for water chemistry, it was difficult to fully submerge probes in this volume.

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several days prior to and

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The test protocol described below is based on what was learned during an initial larval oyster toxicity test performed at the EPA Manchester Environmental Laboratory, Port Orchard, WA using only laboratory control water from the NOAA Manchester Research Station laboratory in Port Orchard, WA. This control water is withdrawn from Little Clam Bay, then filtered and sterilized prior to use as a control. EPA identifies reference stations as field locations as representative as possible of what conditions at a test site would be if the test site were substantially free of contaminants. Little Clam Bay does not current support commercial shellfish growers as does Discovery Bay, and thus is not a representative reference area for Discovery Bay. For this work, Dabob Bay, where oyster growth and development is currently occurring without the toxicity observed at Port Discovery Seafarms has been identified as an acceptable reference site.

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reference area water

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, water temperature

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A minimum of 100 mL of control, reference or test water in glass beakers is needed in order to perform the daily chemical monitoring.

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laboratory room,

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Setting the room temperature of the parasitology lab at Manchester to 20°C worked fine for maintaining temperature, no need for a water bath or environmental chamber.

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water temperature,

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reference area water

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salinity adjustment,

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Unless we get unlucky and hit Port Discovery Seafarms on a day when their ambient salinity is around 10 parts per thousand.

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any

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future

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, water temperature

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So as not to disturb test animals, this should be done in beakers without animals, but which are otherwise handled and treated the same as beakers with animals.

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glass

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frosted white polystyrene

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to 100

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30 to 50

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40

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temperature controlled laboratory room,

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, Coast Seafoods in Quilcene, WA

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or plastic bottles (a maintenance container)

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glass container

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12 mL frosted white polystyrene cup

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e number of larvae in th

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to the desired 10 larvae per exposure chamber. The purposes of this intermediate container is to facilitate accurate counting of the number of larvae exposed to test waters, and to minimize handling stress on the test organisms

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suspension density to 1500-3000 larvae/mL

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glass

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maintenance

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1500-3000 larvae in 1

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10 larvae in 3

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precision 0.5

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3.0

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(e.g. Eppendorf)

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be between 15 and 30 larvae

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not exceed one larvae

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of test solution

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Add control, reference or test water to the 50 mL polystyrene cups as needed to bring the total water volume to 40

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Introduction of 0.5

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. This will result in

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of the larval stock suspension into between 30 and 50 mL of test solution will result in

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between 15 and 50

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one

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time

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Each test, control and reference sample will have a minimum of two replicate sets of eight test chambers per sample.

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F. Count all larvae in each of the eight laboratory control test chambers set up for determining mean larval density and variation. Return these to the test for later examination for survival and settlement in the controls.

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, water temperature

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continue

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may be continued

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as

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if

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for

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to determine if

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has started to successfully occur

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JH. Count the number of live and dead larvae and juveniles (i.e. settled and attached oysters are called juveniles or spat in this procedure) under a dissecting microscope, and record the number of each in each test chamber.

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formalin

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hot water

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This thermal shock will kill surviving larvae, and will prevent any live test larvae from being inadvertently discharged to receiving waters.

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J. Count the number of live and dead larvae and juveniles (i.e. settled and attached oysters are called juveniles or spat in this procedure) under a dissecting microscope, and record the number of each in each test chamber.

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J. Count the number of live and dead larvae and juveniles (i.e. settled and attached oysters are called juveniles or spat in this procedure) under a dissecting microscope, and record the number of each in each test chamber.

KJ. Count the number of live and dead larvae and juveniles (i.e. settled and attached oysters are called juveniles or spat in this procedure) under a dissecting microscope, and record the number of each in each test chamber.

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J. Count the number of live and dead larvae and juveniles (i.e. settled and attached oysters are called juveniles or spat in this procedure) under a dissecting microscope, and record the number of each in each test chamber.

J

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, water temperature

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Determine if larval survival and the conditions under which the toxicity test was performed meets the test acceptability criteria presented in Table 1. If so,

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to determine if survival and/or setting rate significantly differ between test samples and the control and reference samples

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Statistical testing procedures are described in the data quality objectives document for this work.

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A summary of test conditions and test acceptability criteria is listed in Table 1.

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- 100

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30 - 50

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40

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50 - 30

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larval

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complete

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begins,

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maximum exposure

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15. Feeding regime:	*Isochrysis galbana*, added daily to achieve nominal 80,000 algal cells / mL test solution

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and reference sample

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70

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50

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normal

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survival of settled

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e

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l settlement

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surviving

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and reference sample

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Header and footer changes

Text Box changes

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Footnote changes

Endnote changes